

TEMPERATURE AND CRITICAL ILLUMINATION FOR REACTION TO FLICKERING LIGHT

II. SUNFISH

By W. J. CROZIER, ERNST WOLF, AND GERTRUD ZERRAHN-WOLF

(From the Biological Laboratories, Harvard University, Cambridge)

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I

The relationship between frequency of interruption (F) and intensity of illumination (I) for threshold response indicating discrimination of non-continuous light (flicker) exhibits in the case of the sunfish, as in the human (Hecht and Verrijp (1932-33 *a, b*); Hecht (1934); Hecht, Schlaer, and Smith (1935)), two distinct sections: a rather flat low intensity portion assigned to the exclusive or predominant activity of rods, and a steeper portion, rising abruptly from the former, reflecting the involvement of cone vision (Wolf and Zerrahn-Wolf (1935-36); Crozier, Wolf, and Zerrahn-Wolf (1936-37 *a*)). The curve of I_m as a function of F differs from that of F_m as a function of I in a manner predictable on the basis (Crozier (1935-36)) that the law of the relationship is properly to be described not by a curve, but as a band based upon the observable dispersions of the measurements (Crozier (1935-36); Crozier, Wolf, and Zerrahn-Wolf (1936-37 *a*)). The curve of I_m as a function of F is quite well fitted, however, by the logistic

$$F = F_{max.}/(1 + e^{-\rho \log I}), \quad (1)$$

where the value of ρ differs for the rod and cone sections; or by a log probability integral; or at 21.5° by another form of this equation, with theoretical implications, which has been used to describe the flicker extinction curve in the case of man (Hecht, Schlaer, and Smith (1935)):

$$K I = \frac{F^n}{(F_{max.} - F)^m}. \quad (2)$$

The equation does not really fit in a satisfactory way at the extreme lower (rod) end. The question arises as to whether this stationary state equation ((2), with $F \equiv x$, the stationary state concentration of photoproduct derived from decomposition of photosensitive material) correctly formulates the theory of the mechanism whereby flicker extinction is attained. There should be no essential difference in the properties of data based upon (a) marginal recognition of flicker and (b) upon marginal extinction of flicker.

The K in equation (2), on the basis that (2) is the stationary state equation, is the ratio of two velocity constants (Hecht, Shlaer, and Smith (1935)),

$$K = 2k_1/k_2;$$

k_1 = the photochemical velocity constant concerned with the production of x , k_2 = the velocity constant of the thermal "dark" process whereby x is removed.

Equation (2) thus requires that with increase of temperature k_2 will increase much faster than k_1 ; and consequently that if F_{max} is not affected by temperature then at any fixed F the mean critical illumination must increase.

We have tested this in the case of the larvae of the dragon fly *Anax junius*. Instead of increasing with rise of temperature, I_m decreases. This result is quite consistent, however, with the requirements of the idea that a certain type of difference between the effect of a light flash and the after effect of its action is required for recognition of flicker (Crozier (1935-36); Crozier, Wolf, and Zerrahn-Wolf (1936-37 b, c)), and was indeed predicted by the use of this conception; it also gives a reasonable account of the properties of the variation of I_1 . It has been desirable to extend these observations to deal with the visual reactions of a vertebrate. If the same type of dependence of I_m and of σ_{I_1} upon temperature should be apparent, as is the case, the possibility of a general theoretical treatment is thereby greatly improved. And the real meaning of equation (1) and particularly of equation (2), will be clearer if in the case of the vertebrate the rod and cone portions of the flicker curve (with their necessarily different magnitudes of K) are found to be influenced by change of the temperature in just the same manner or in different ways.

II

The experiments were made with the sunfish previously called *Lepomis, Enneacanthus gloriosus* (Holbrook), using exactly the procedure in our earlier work. We are indebted to Mr. W. C. Schroeder of the Museum of Comparative Zoology for the identification of these "little sunfish" and their separation from the very closely similar *Lepomis*. We had previously determined I_m as a function of F at $21.5^\circ \pm 0.5^\circ$ (Wolf and Zerrahn-Wolf (1935-36)). This curve was re-determined

TABLE I

Mean rank-order positions (R_I), indicating mean comparative values of I_1 in a series of tests at various values of F , for different individual sunfish; R_F = a series based on determinations of F_m ; the same individuals were used in all tests at 12.4° and 27.3° , another lot at 21.5° . Increasing R_I in order of increasing I_1 .

| Sunfish No. | Temperature | | | |
|------------------------------|---|------------------------|------------------------|---|
| | 12.4° | 21.5° | | 27.3° |
| | $N = 10$ fish R_I $n = 26$ sets | $N = 12$ fish | | $N = 10$ fish R_I $n = 18$ sets |
| | | R_F $n = 23$ sets | R_I $n = 15$ sets | |
| 1 | 6.13 | 4.55 | 6.57 | 5.94 |
| 2 | 5.87 | 4.69 | 4.87 | 6.88 |
| 3 | 6.13 | 5.14 | 7.40 | 6.53 |
| 4 | 6.75 | 6.86 | 7.10 | 6.14 |
| 5 | 5.27 | 6.76 | 7.87 | 5.03 |
| 6 | 4.02 | 6.76 | 7.30 | 5.28 |
| 7 | 5.50 | 8.42 | 4.20 | 4.97 |
| 8 | 4.61 | 6.76 | 5.33 | 3.92 |
| 9 | 5.17 | 8.01 | 7.47 | 5.50 |
| 10 | 5.15 | 5.76 | 7.07 | 4.47 |
| 11 | | 6.91 | 6.03 | |
| 12 | | 7.12 | 7.23 | |
| Mean \pm P.E. ₁ | 5.46 ± 0.549 | 6.48 ± 0.846 | 6.24 ± 0.915 | 5.47 ± 0.652 |

at $12.4^\circ \pm 0.4^\circ$ and at $27.3^\circ \pm 0.3^\circ$. These temperatures were chosen for convenient comparison with the results given by *Anax* larvae at the same temperatures (Crozier, Wolf, and Zerrahn-Wolf (1936-37c)). Several points on the curve at 21.5° were re-determined, and found to agree very precisely with the figures in the earlier series.

Ten individuals were employed, the same fish being used throughout the tests. With each fish, at each flicker frequency (F), three readings of critical intensity (I_c) were made; the average of these 3 = I_1 for this individual. From the 10 values of I_1 at each F the mean (I_m) was computed and the P.E. of I_1 .

The procedure was that which we have already described (Wolf and Zerrahn-Wolf (1935-36); Crozier, Wolf, and Zerrahn-Wolf (1936-37*b*, *c*)). We desired to see how the variation of I_1 would behave at the several temperatures. The variation of F_1 is less easy to study in this respect, since the law of σ_F as a function of fixed values of I is not so simple as that of σ_I as a function of I_m (*cf.* Crozier (1935-36); Crozier, Wolf, and Zerrahn-Wolf (1936-37*a*, *b*)). Our use of $P.E._{I_1}$ is based upon the fact that, as I_1 has been determined, it reflects in our population of individuals a fluctuating capacity to exhibit the index response. The relative sensitivities of the 10 individuals are randomly distributed in the successive tests at different values of F ; this is taken to indicate that each fish gives, at any time, an independently random level of I_1 ; the several individuals exhibit, at one time, levels of sensitivity such as any one fish would show if examined repeatedly; in combination with the lawful behavior of $P.E._{I_1}$ as a function of I_m we are thus

TABLE II

Showing the absence of correlation between relative sensitivities of individual sunfish in successive sets of determinations of I_1 . The individual rank-order numbers (R_I) are assigned in order of increasing values obtained for I_c . The mean values of $R_{I''}$ in the second of two determinations (same afternoon) associated with $R_{I'}$ classes in the first determinations were:

| $R_{I'}$ | 1.5 | 3.5 | 5.5 | 7.5 | 9.5 |
|---------------------------------------|------|------|------|------|------|
| 12.4° (24 sets; 10 fish) | 5.72 | 6.25 | 5.30 | 5.38 | 4.75 |
| $R_{I''}$ 21.5° (23 sets; 12 fish) | 6.47 | 7.01 | 5.80 | 6.93 | 6.80 |
| 27.3° (18 sets; 10 fish) | 4.94 | 5.41 | 5.21 | 5.61 | 5.56 |

enabled to regard the indices of dispersion as measuring a primary property of the capacity to give the response to flicker. This has been indicated already for our measurements at 21.5°. It is substantiated by the data at the other two temperatures, as Table I demonstrates. It is apparent in Table I that the average position of an individual, as regards relative sensitivity, does not differ by more than is to be expected by chance from the mean of its set, or from that of any other individual; the extreme difference between entries in any column of Table I = 2.3 $P.E. diff.$. In successive sets of trials on the same day (Table II) there is no correlation between the two rank-order numbers for the same individual.

III

The data are given in Table III; the measurements of I_m at 21.5° (Wolf and Zerrahn-Wolf (1935-36); Crozier (1935-36); Crozier, Wolf, and Zerrahn-Wolf (1936-37*a*)) are included for comparison. They

are exhibited graphically in Fig. 1. The range of intensities covered is so great that in a plot upon this scale it is difficult to do justice to the figures.

TABLE III

Mean critical intensities ($\log I_m$, and P.E. I_1) for reaction of sunfish to flickering light, as a function of flicker frequency F , at three temperatures. (Data at 21.5° from: Wolf and Zerrahn-Wolf (1935-36); Crozier, Wolf, and Zerrahn-Wolf (1936-37a). See text.

| F per sec. | $\log I_m, \pm \text{P.E. } I_1, \text{ millilamberts}$ | | | | | |
|--------------------|---|--|---|--|---|--|
| | 12.4° | | 21.5° | | 27.3° | |
| 3 | $\bar{7}.9201 \pm 0.536 \times 10^{-7}$ | | $\bar{7}.6555 \pm 0.694 \times 10^{-7}$ | | | |
| 4 | $\bar{6}.3392 \pm 0.106 \times 10^{-6}$ | | $\bar{6}.1784 \pm 0.520 \times 10^{-6}$ | | $\bar{7}.9708 \pm 0.372 \times 10^{-7}$ | |
| 5 | $\bar{6}.5594 \pm 0.154 \times 10^{-6}$ | | $\bar{6}.3701 \pm 0.399 \times 10^{-6}$ | | $\bar{6}.1775 \pm 0.378 \times 10^{-7}$ | |
| 6 | $\bar{5}.3593 \pm 0.211 \times 10^{-5}$ | | | | | |
| | $\bar{5}.3784 \pm 0.931 \times 10^{-6}$ | | $\bar{5}.2385 \pm 0.303 \times 10^{-5}$ | | $\bar{5}.0253 \pm 0.281 \times 10^{-6}$ | |
| 7 | $\bar{4}.3738 \pm 0.165 \times 10^{-4}$ | | $\bar{4}.1855 \pm 0.279 \times 10^{-4}$ | | $\bar{5}.9937 \pm 0.217 \times 10^{-5}$ | |
| 8 | $\bar{3}.1844 \pm 0.357 \times 10^{-4}$ | | $\bar{4}.9954 \pm 0.190 \times 10^{-3}$ | | $\bar{4}.7711 \pm 0.133 \times 10^{-4}$ | |
| 9 | $\bar{2}.0060 \pm 0.152 \times 10^{-3}$ | | | | | |
| | $\bar{2}.0047 \pm 0.361 \times 10^{-3}$ | | | | | |
| | $\bar{2}.0132 \pm 0.624 \times 10^{-3}$ | | $\bar{3}.7983 \pm 0.124 \times 10^{-2}$ | | $\bar{3}.6732 \pm 0.274 \times 10^{-3}$ | |
| 10 | $\bar{2}.7638 \pm 0.175 \times 10^{-2}$ | | $\bar{2}.5600 \pm 0.743 \times 10^{-2}$ | | $\bar{2}.3565 \pm 0.846 \times 10^{-3}$ | |
| 12 | $\bar{2}.9560 \pm 0.339 \times 10^{-2}$ | | | | $\bar{2}.5419 \pm 0.124 \times 10^{-2}$ | |
| 15 | $\bar{1}.1735 \pm 0.239 \times 10^{-2}$ | | | | | |
| | $\bar{1}.2375 \pm 0.146 \times 10^{-1}$ | | $\bar{2}.9543 \pm 0.126 \times 10^{-1}$ | | $\bar{2}.7440 \pm 0.236 \times 10^{-2}$ | |
| 20 | $\bar{1}.4637 \pm 0.160 \times 10^{-1}$ | | $\bar{1}.2591 \pm 0.177 \times 10^{-1}$ | | $\bar{1}.0592 \pm 0.319 \times 10^{-2}$ | |
| 25 | $\bar{1}.7755 \pm 0.268 \times 10^{-1}$ | | $\bar{1}.5631 \pm 0.630 \times 10^{-1}$ | | $\bar{1}.3609 \pm 0.166 \times 10^{-1}$ | |
| | | | | | $\bar{1}.3602 \pm 0.715 \times 10^{-2}$ | |
| 30 | $0.0453 \pm 0.278 \times 10^{-1}$ | | $\bar{1}.8118 \pm 0.731 \times 10^{-1}$ | | $\bar{1}.6249 \pm 0.162 \times 10^{-1}$ | |
| 35 | 0.3465 ± 0.120 | | 0.1418 ± 0.129 | | $\bar{1}.9444 \pm 0.291 \times 10^{-1}$ | |
| 40 | 0.7308 ± 0.168 | | | | | |
| | 0.7383 ± 0.151 | | 0.4601 ± 0.281 | | $0.2423 \pm 0.620 \times 10^{-1}$ | |
| 45 | 1.2748 ± 0.571 | | | | | |
| | 1.2728 ± 0.465 | | 1.0465 ± 0.415 | | 0.8532 ± 0.166 | |
| 48 | | | | | 1.3056 ± 0.558 | |
| 50 | 2.2751 ± 1.844 | | | | | |
| | 2.2817 ± 1.933 | | 2.2264 ± 11.53 | | 2.1898 ± 2.994 | |

We have indicated that the measurements at 21.5° are described rather well by the equation (Hecht, Schlaer, and Smith (1935)):

$$KI_m = F^n / (F_{max} - F)^m \quad (2)$$

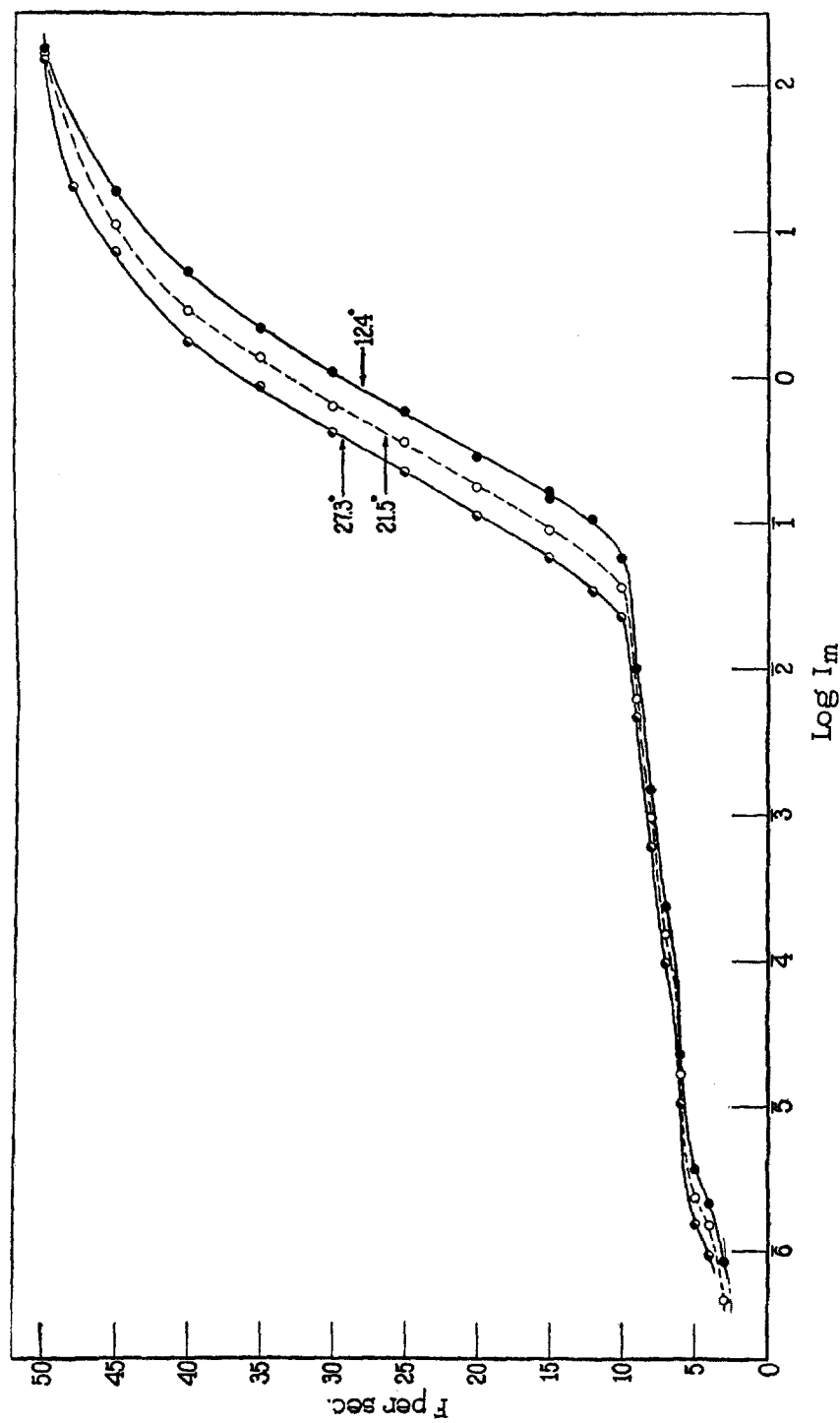


FIG. 1. Mean critical illumination, as $\log I_m$, for response of sunfish to flicker at various flicker frequencies F , at three temperatures.

where $n = 2$, $m = 2$ for the lower (rod) segment and $n = 2$, $m = 1$ for the upper (cone) portion (Crozier, Wolf, and Zerrahn-Wolf (1936-37 b)). The extent to which this is true of the data at the other two temperatures (Fig. 2) may be most conveniently shown in a plot of $\log F$ vs. $\log I_m$. The fit may be considered acceptable for the "cone portion," although with these values of the exponents it is not really adequate at 12.4° or at 27.3° ; the curvature is a function of temperature. The deviation at the lowest frequencies is real and significant.

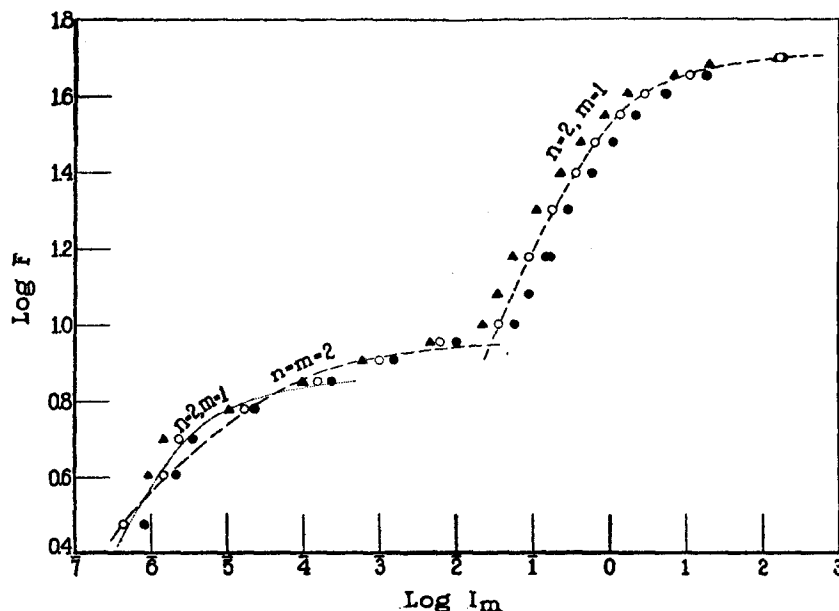


FIG. 2. $\log I_m$ as a function of $\log F$. See text.

(The arrangement of the data at the lower end has earlier been treated as due to chance variation (Crozier, 1935-36).) On the basis of a possible separation of the rod and cone contributions which is discussed subsequently (page 424), the region from $F = 7$ to $F = 10$ represents a zone of intensities in which the two contributions are added; one could then fit equation (2) to the points at $F = 3$ to $F = 7$ (21.5°) with $n = 2$, $m = 1$ as in the case of the cone section (dotted line in Fig. 2). The deviations from this at the other two temperatures are similar to those in the case of the cone segments. The devia-

tions appear to be systematic, and are also found in a series of measurements in which F_m was determined at fixed I (Crozier, Wolf, and Zerrahn-Wolf (1936-37 *a*)). They *may* be due to the use of "neutral" filters with slightly different properties in obtaining the low intensities of illumination; the intensities at $F = 3, 4, 5$ were gotten with a 1:10,000 filter, at $F = 6$ a 1:1,000, at $F = 7, 8$ a 1:100, at $F = 9$ a 1:10 filter. The variation data, however (Fig. 4), contain no indication of qualitative differences.

It is to be noticed (1) that as the temperature is lowered I_m is increased at constant F , and (2) by about the same proportion at all intensities until the maximum F is approached; (3) that the change in I_m is proportionately about the same for both rod and cone sections of the curve; and (4) that the maximum F is independent of temperature and practically independent of intensity. Points (1), (2), and (4) agree precisely with those already established for the flicker response of *Anax*. We have discussed the way in which these phenomena appear to support the "intensity discrimination" conception of recognition of flicker (Crozier, Wolf, and Zerrahn-Wolf (1936-37 *c*)). We need only call attention here to the fact that the similar shift of the rod and of the cone portion of the curve (Fig. 1) is also consistent with this view. Since in equation (2) m (at 21.5°) may be taken to differ for the rod and cone sections, and since the k_2 's might not therefore (or in any case) be expected to have the same temperature coefficients, we might not expect that if the theory leading to this equation were applicable change of temperature would lead to the same kind of shift in the positions of the two portions of the entire curve. But if we deal with the operation of a mechanism of discrimination, based upon the involvement of a decay process governing the after effect of a light flash, this is not altogether unexpected. The constancy of $F_{max.}$ at different temperatures, and the inconstant curvature of the upper portions of the $F - I_m$ curves, leads one to expect that equation (2) could not apply unless the exponents n and m (apparent orders of reaction in equation (2)) are functions of temperature. Fig. 2 shows that the equation used for the curve at 21.5°, with $n = 2$, $m = 1$ for the cone portion, $n = 2 = m$ for the rods, does not really give a fit for the cones at the other two temperatures. Plotted in terms of equation (1), on a logistic grid (the modulus has been neglected), the exponent ρ is prac-

tically constant and is independent of temperature (Fig. 3). The *form* of the function is not affected by temperature; its parameters therefore measure a property of the reacting organism, and make possible an investigation of the nature of this property.

It is of some interest to consider briefly the difficulties arising if one attempts to estimate temperature characteristics for the underlying processes which might be presumed to be involved in determining the

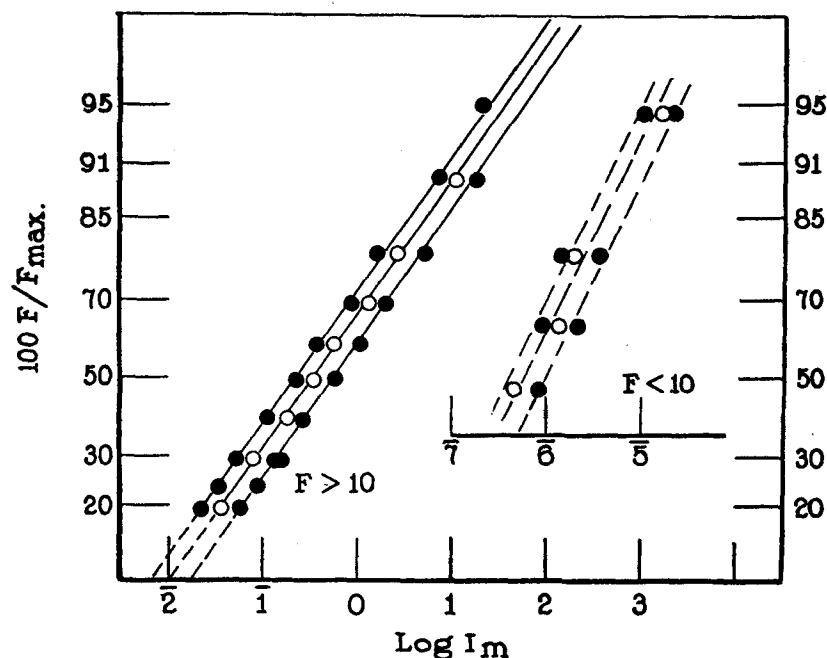


FIG. 3. The data of Fig. 1 on a logistic grid: $F/F_{max} = 1/(1 + e^{-\rho \log I})$, with $\rho = 1.37$ for the cone portion ($F > 10$), $\rho = 0.5$ for $F < 10$.

effects shown in Fig. 1. As in the case of *Anax* (Crozier, Wolf, and Zerrahn-Wolf (1936-37 c)), the temperature coefficient of intensity at constant F is negative and is a function both of F and of temperature (T). This is scarcely consistent with the idea that a constant amount of photoproduct is involved in the light-dark cycle at each F and at constant temperature. I_m is a declining and practically rectilinear function of $t^\circ\text{C}$., the slope constant being a function of F . The temperature coefficient of F ($= 1/2t$) at constant I is positive, and is a

function of I and of T ; its values differ for the rod and cone sections, and are not the same as that for I at constant F . The conclusion necessarily is that the immediate basis for recognition of flicker is quantitatively a function of F , of I , and of T .

IV

The connection between I_m and P.E. I_1 was discussed in considering the sunfish flicker data at 21.5° (Crozier (1935-36); Crozier, Wolf, and Zerrahn-Wolf (1936-37 a)). It was pointed out that for the lower section of the curve these two quantities are rectilinearly proportional. Above a certain intensity the logarithmic plot of I_m vs. P.E. I_1 , however, may be regarded as rectilinear but with a slope (exponent) = 1. A slope of 1 was obtained for this upper portion of the curve by plotting \log P.E. I_1 against $\log (I + 0.10)$. The idea was tentatively entertained that this might signify, for the cone section of the curve, the necessity for a subtraction of the variability due to the continuing participation of rod excitation. This notion must now be somewhat revised. At the other two temperatures used the same type of connection between I_m and P.E. I_1 is again observed, but with a lateral shift in the position of the graph and a change in its breadth (Fig. 4).

The shift of position is such that the lines for 12.4° and for 27.3° are to the right of those for 21.5°. The best fitting central line has, for the lower portion, the equation

$$\begin{aligned} \text{P.E. } I_1 &= I_m(0.182) \text{ at } 21.5^\circ. \\ &= I_m(0.0314) \text{ at } 27.3^\circ. \\ &= I_m(0.0562) \text{ at } 12.4^\circ. \end{aligned}$$

At given intensity P.E. I_1 increases in the order 27.3°, 12.4°, 21.5°, while F increases directly as T increases; at given F , P.E. I_1 goes through a maximum at 21.5°. This is precisely the relationship encountered in the case of the experiments with *Anax* (Crozier, Wolf, and Zerrahn-Wolf (1936-37 c)). If one considers P.E. I_1 as a function of F , curves are obtained of the same general character as for I_m as a function of F (Crozier (1935-36)), but the order of increasing P.E. I_1 at the three temperatures puts the curves in the sequence 27.3°, 12.4°, 21.5°.

These facts substantiate the lawful character of the behavior of P.E. I_1 as a function of intensity. They are quite inconsistent with any idea to the effect that P.E. I_1 measures an "error of experiment;" the

precision of the mere judgment of the occurrence of the index-response depends on the sharpness and clarity of the animal's movements; this increases with increasing I and with elevation of temperature and should lead to a decrease of $P.E._{I_1}$, but there is no correlation of this sort.

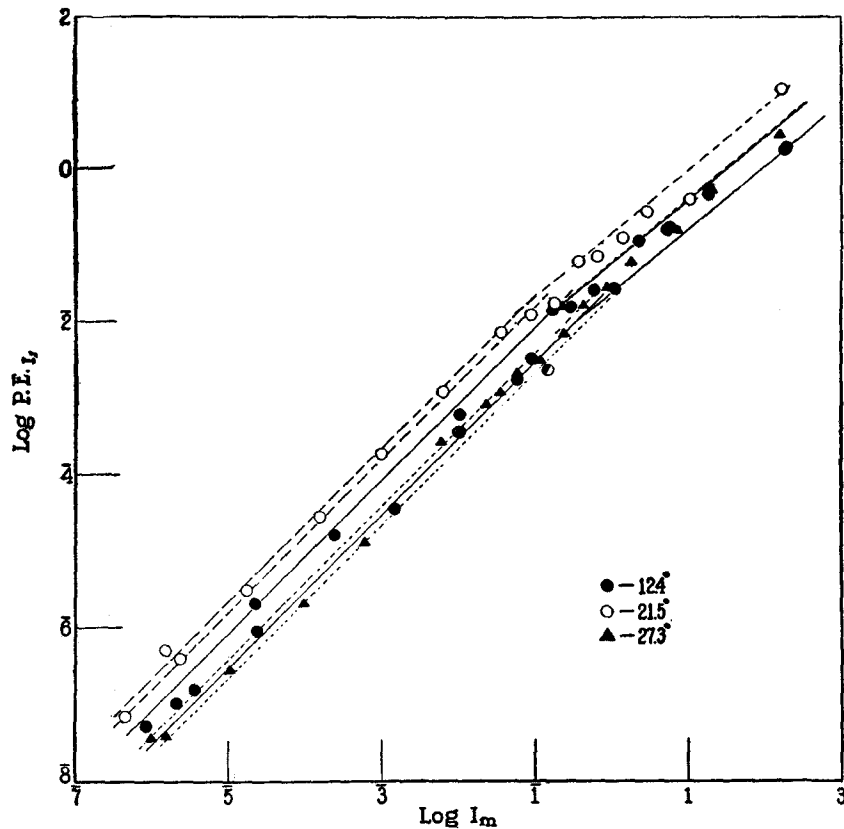


FIG. 4. The variability of I_1 for response to flicker: $\log P.E._{I_1}$ vs. $\log I_m$. See text. The slope up to $\log P.E._{I_1} = 2.1$ is 1. The vertical breadth of the plot is a function of temperature. The position of the graph is moved laterally as a function of temperature.

This may be investigated by a procedure already used in principle for the analysis of variation of geotropic behavior of rats (Crozier and Pincus (1931-32a and b); (1932-33); (1934-35)). In those instances variation of performance was measured, not variation of intensity giving threshold performance. The principle,

however, is that the relation between variation and mean may be of the same form, although the variation index at a given level of the inciting variable may be changed by altering conditions. On one particular day, for reasons which are obscure, all our fishes used in the present experiments were unusually "restless" and gave quite violent responses to flicker at the thresholds for reaction. The mean intensities obtained on this day, at 12.4°, were:

| F | $\log I_m$ | $P.E._{I_1}$ |
|-----|------------|--------------|
| 40 | 0.7340 | 0.424 |
| 50 | 2.2774 | 7.146 |

Reference to Table III shows that these values of I_m each agrees admirably with the results of two other determinations spaced some days apart, and in fact are indistinguishable from the means of these pairs. But $P.E._{I_1}$ was 2.5 to 3.8 times as great as in the sets obtained from the same individuals when not in this excited state. The slope of the line connecting $\log P.E._{I_1}$ with $\log I_m$, however, agrees precisely with that given by the acceptable measurements. It will be of some importance to investigate the action of general excitatory conditions upon the properties of $P.E._{I_1}$, since this may permit a quantitative separation of the rôle of variation due to the reacting mechanism from that due specifically to the mechanism of intensity discrimination. We suggest in another connection (page 430) the importance of a genetic test of the character of the flicker curve which should also give information as to the functional basis of the variability.

We have already indicated how the changes in variability appear to be consistent with a theory of response to flicker (Crozier, Wolf, and Zerrahn-Wolf (1936-37 c)), in the case of the *Anax* data, and this need not be repeated here. Further data leading to a more extensive test of the "intensity discrimination" conception will be given by experiments in which the relation of light time to dark time is systematically altered.

A noteworthy additional feature of the data has to do with the spread of the band encompassing or describing $P.E._{I_1}$ as a function of I_m . As found with *Anax*, the "spread coefficient" of $P.E._{I_1}$, which may be taken as B in the formula

$$P.E._{P.E._{I_1}} = B P.E._{I_1} / \sqrt{N}$$

is also a function of temperature. The scatter of the determinations of $P.E._{I_1}$ is greater at the lowest temperature. This may be estimated from $\sqrt{N}(P.E._{I_1}'' - P.E._{I_1}')/P.E._{I_1}$ at fixed I_m , where $P.E._{I_1}''$ is the upper limit of the band, $P.E._{I_1}'$ the lower limit. Approximate values for this fraction are: 12.3°, 3.32; 21.5°, 0.906; 27.4°, 1.77, for the lower

section of the graphs in Fig. 4; for the upper section the proportionate spread is: 2.66; 3.05; 1.84. These values are better established at 12.3° and at 27.4° than at 21.5°, and the latter series was made with a different series of individuals; a larger number of determinations in the rod region at 21.5° would undoubtedly give a wider spread. The

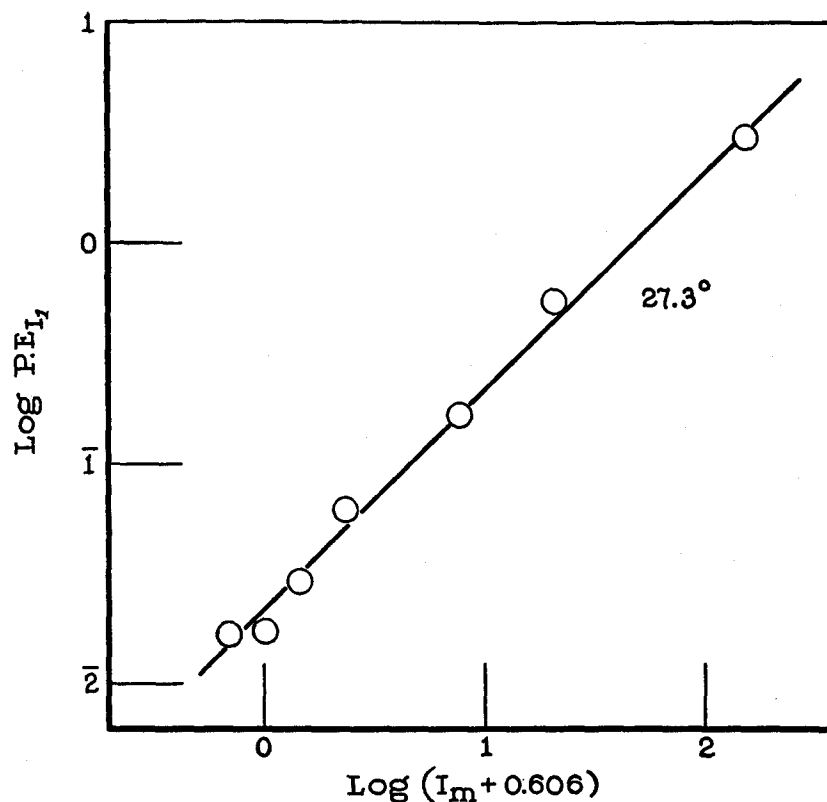


FIG. 5. Log P.E. I_1 vs. log ($I_m + c$), for values of P.E. $I_1 >$ antilog 2.1, to give a slope = 1.

general implication, as in the *Anax* case, is to the effect that the spread coefficient for P.E. I_1 is less the higher the temperature, corresponding to an increase in the speed (hence "precision") of the processes leading to reaction when the excitation is adequate.

The change of slope of the logarithmic graph of P.E. I_1 against I_m occurs at a higher value of I_m in the order 21.5°, 12.4°, 27.3°, but the

level of P.E. r_1 at which the transition is apparent is substantially the same (antilog 3.9). The upper portions of the bands can be described as straight, with slope = 0.83 (Fig. 4), or, by addition of a constant to I_m , as rectilinear with a slope of 1 (Fig. 5). Adding a constant to I_m is equivalent to subtracting a constant (0.00745) from P.E. r_1 . We have suggested (Crozier, Wolf, and Zerrahn-Wolf (1936-37 c)) that this might signify the correction for "full excitation of rods." The values of log I_m at which the correction for this purpose becomes necessary are approximately: 12.4°, 1.15; 21.5°, 2.5; 27.3°, 1.5. These levels show no exact correspondence with the order of positions of the transitions to the cone portions of the flicker curves (Fig. 1); the out-of-place value is that at 27.3°. If we suppose, however, that the approximately constant level of log P.E. r_1 = 3.85 corresponds to the full effect of excitation of rods, we can perhaps believe that with lower absolute values of P.E. r_1 at given I_m , this contribution to the total variation should be more effective. It is possibly not without significance that the two determinations at $F = 25$ (27.3°) and the two at $F = 15$ (12.4°) show a wide difference at precisely the level (Fig. 4) which is assumed to mark transition to the lower slope.

V

A special problem arises in dealing with the flicker measurements with eyes of vertebrates. It has to do with the separate functioning of rods and of cones (Hecht and Verrijp (1932-33b); Hecht, Schlaer, and Smith (1935)), reflected in the two segments of the curves of Fig. 1. This double type of curve is not obtained with animals in which there is no evidence of duplex constitution of the excitable retinal fields, such as *Anax* and the bee (Crozier, Wolf, and Zerrahn-Wolf (1936-37 b); Wolf (1933-34)). The immediate question is, From the curves in Fig. 1 is it possible to deduce properties of the mechanism of excitation? A practical aspect of this concerns the precise mode of superposition of the cone effect upon that assignable to the rods. Two extreme positions are possible: either the response to flicker is determined by the most sensitive excitable elements, or it is due to a critical value of the summated excitation of all the elements excitable under the conditions. For diverse visual functions one or the other view might be necessary. And a still different theoretical position is

possible—if not indeed required. The point has been made that it is inadmissible to deduce a theory of mechanism from measurements of average critical excitation energies alone, since, in view of the variation of performance inherent in biological systems, an essentially arbitrary procedure must be adopted if theory is to be tested by curve fitting (Crozier (1935–36)). The functional connection between flicker frequency and fluctuation of intensity to give the end point is conditioned by the fluctuating effect produced by a given intensity. To assume that this fluctuation is governed by peripheral events, in the eye, is arbitrary and unnecessary. We must deal solely with “effects,” defined in the only manner in which they can be defined, namely in terms of the result by which we recognize them. We have dealt with marginal response to flicker in terms of intensity discrimination, considering that a discrimination must be possible between the effects of flashes of light and the effects of their after action (after image) (Crozier (1935–36)). This view is required by the relation of flicker recognition curves to temperature, and it accounts satisfactorily for the variability functions (Crozier, Wolf, and Zerrahn-Wolf (1936–37 *c*)). The total direct effect of a light flash within a fixed time interval must be the result of the total of nerve impulses to which it gives rise. There is enough general evidence to show that at least two important elements are to be recognized as taking part in this total: the number of active retinal elements, and the number of impulses proceeding from each (*cf.* Hecht (1934)). The particular aspect of performance selected for measurement will determine whether (1) number of active elements, governed by the relation between threshold distribution and intensity, or (2) total density of nervous effect, is the limiting variable. The intensity discrimination view of marginal response to flicker calls for a form of the integrated density of effect idea: the total effect of a flash is just discriminated from the total effect in the interval after the flash. It must be presumed that different nervous elements of any one type (*e.g.*, cones) will vary, at a given time, in the magnitude of the direct effect produced in them by the light flash and in the associated after effect, and consequently in their ability to participate in the activity of discrimination. The phrase “nervous element” is used deliberately, to include peripheral sense cell *and* its central nervous representation; the ultimate decision

as to the effective locus, very possibly central, is not prejudiced by this terminology, and the problems of detail are kept more fairly in view. It is to be conceived that the variation of capacity to participate in the discrimination of flicker, under constant external conditions, is analogous to the change in this capacity produced by altering the temperature. At constant temperature, with increasing I and F , an intensity is ultimately reached beyond which, at still higher intensities, a *longer* time must be allowed for the decay of after effect in the dark interval if the effects in the light and dark intervals are to be just discriminated. This is not to be confounded with the requirements of minimum time for threshold excitation of a sensory cell. Diagrammatically, this may be represented in a manner similar to that used in our preceding paper, in which the action of temperature was considered (Crozier, Wolf, and Zerrahn-Wolf (1936-37 c)). With sufficiently high intensity of flash little change in the decay curve of the after effect is to be produced by further increase in intensity, so that comparison of the areas under the light flash curve and the decay curve, using the criterion of marginal recognition of difference, will require longer time intervals for increases of intensity beyond this point; this means that for a given F , beyond the maximum, as I increases a smaller and smaller number of excitable elements will be able to contribute to the recognition of flicker.

Precisely this finding has been made at the upper end of the flicker curve for man, with small test fields (Hecht and Verrijp 1932-33 a)); the curve bends, and the function is no longer monotonic. With bee, *Anax*, and sunfish this would in all probability be found if, with our apparatus, sufficiently precise measurements could be made with much higher intensities than we are able to use. The drop in the cone curve is pushed to higher intensities when a flickered area is surrounded by a non-flickering field (Hecht, Schlaer, and Smith (1935)); when the whole eye is exposed to flicker the failure of rods to be involved at high intensities may in effect provide a kind of steady surround at the periphery of the retina; this may result in the absence of a drop in the curve for the whole eye until very high intensities are reached. Under proper conditions its counterpart might well be detectable in connection with the rod curve. Certain human flicker curves, but not all, do show a significant depression at or slightly before the onset of the

cone rise (Ives (1912); Hecht and Verrijp (1932-33 *a*)), which tends to be more extensive in the case of retinal areas further toward the periphery and (data of Lythgoe and Tansley (1929)) with more complete light adaptation. In the data on the sunfish (Fig. 1) there is no dip, but a distinct and significant upward trend starting at about $\log I_m = 4.0$ or above. This is shown in all four independent sets of measurements (*cf.* also Crozier, Wolf, and Zerrahn-Wolf (1936-37 *a*)). The beginning of this rise is at progressively higher intensity as the temperature falls (Fig. 1). It provides anchor points by which to judge the results of an attempt at downward extrapolation of the cone

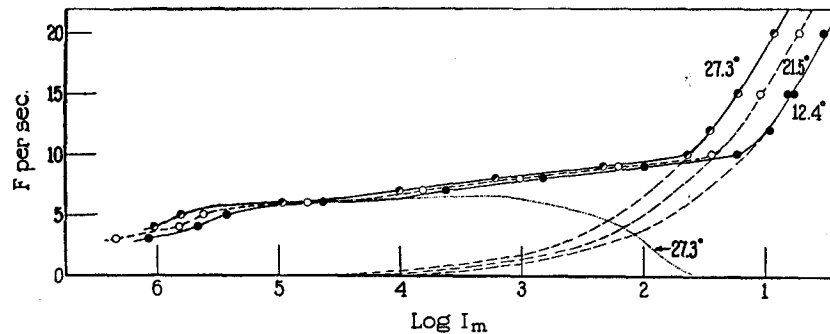


FIG. 6. The result of extrapolating the logistic curves for the cone portions of the flicker response curves (Fig. 3). The extrapolation cuts the $\log I_m$ axis at each temperature at a point appropriate to account for the rise near $\log I_m = 4.0$ in Fig. 1. The difference curve (dotted) is discussed in the text (only that for 27.3° is shown).

portion of the curve. Any such extrapolation is somewhat dubious at best; its outcome is to be evaluated by the usefulness and coherence of its predictions. The rectilinear character of the cone curve on a logistic grid (Fig. 3), down to $F = 10$, invites backward extrapolation to lower flicker frequencies by means of this relationship. The extent of the extrapolation is only 20 per cent of the total range of F . The outcome, shown in Fig. 6, indicates that the extrapolated origin of the cone curve comes at a value of I_m which at each temperature coincides with the location of the "bump" on the original $F - \log I_m$ curve. The logistic plot of F_m as a function of I at 21.5° (data in: Crozier, Wolf, and Zerrahn-Wolf (1936-37 *a*)) agrees precisely with that for

I_m at this temperature. Accepting this extrapolation we may then determine by difference the contribution made by rod excitation at flicker frequencies $< F = 10$ to the total effect upon which response to flicker is based. Above $F = 6.3$, a level apparently not influenced by temperature (just as with the cone maximum), this contribution declines. The decline is associated with higher values of I_m the lower the temperature. This is consistent with our view that as the temperature is lowered the velocity constant for after effect decay is less, as then at a given F a higher intensity should be permissible if a given kind of difference is to be obtained between effect of light flash and its decay. We therefore regard the results of this analysis as consistent with the indications provided by the human flicker data. They lead to the view that in the region of intensities where both rods and cones function to contribute to the effect determining response to flicker the contributions of the two are additive, but that the rod contribution fades out of the picture as higher and higher intensities (and therefore higher flicker frequencies for the cone curve) are used. On this basis also the photostationary state character of the shape of the flicker curve is clearly illusory.

This mode of separation of rod and cone curves involves arithmetic addition of flicker frequencies in the region of overlapping, a procedure which raises a number of questions. The logistic formulation of the cone curve implies that critical flicker frequency measures an additive property of some feature of excitation which is governed by $\log I$. Why it should be so is another matter. A suggestion is given by data on critical flicker frequency as a function of area of retina. The determinations of the flicker curve for equal areas at various locations on the human retina show that F increases as the total number of active elements involved at the given intensity becomes greater (Hecht and Verrijp (1932-33 *a*)). The relation between F and wave length is of the same kind (Hecht, Schlaer, and Smith (1935)). At a constant I , F increases as the area is made greater at a given location (Granit and Harper (1930)). For the range of intensity where F is very nearly proportional to $\log I$, it is nearly proportional to $\log \text{area}$ at fixed I . If increase of area at fixed I means increase in number of elements of effect, and increase of I with constant area has the same sort of significance, then the relationship between them (at least over

this range) is expressible as a simple power function. In equation (1) we can then write

$$F = F_{max.}/(1 + e^{-h \log cN}),$$

where h and c are constants and N signifies the variable by means of which the number of active elements of the effect determining F is increased. For N may be inserted either *area* (A) or *intensity* (I), on the basis that $A \equiv q I^s$. Essentially this situation has been pointed out by Hecht: altering the area of an excited field merely shifts the position of the $\log F$ vs. $\log I$ curve without changing its shape (Hecht, Schlaer, and Smith (1935)). Wald¹ has been able to show that the dependence of various visual functions upon area is quite satisfactorily accounted for on the assumption of a population distribution of excitabilities as a function of $\log I$. Accepting this, F corresponds simply to number of excitation elements entering into the determination of critical flicker. Its additive character in the case of contributions from rods and from cones is therefore not mysterious. Since F has the dimensions of a frequency, we must consider that the speed of the process as a whole, due to the totality of excitation given by equal light and dark intervals (*i.e.*, its central nervous representation), which achieves reaction to flicker depends upon and is directly proportional to the number of excitation elements (units of excitation) involved. This is a reasonable result despite the complexity of the comparisons apparently concerned in the intensity discrimination which we have conceived to be basic to the response to flicker. It is consistent with the relationship of F to temperature: at fixed I , F increases as T increases.

In another way the additive property of critical flicker frequency is also shown by its behavior at various retinal locations during the progress of dark adaptation, in the data of Lythgoe and Tansley (1929). The evidence there given indicates addition of the effects respectively due to rods and cones as these are changing during adaptation: in the fovea, F at given intensity falls as dark adaptation proceeds, with declining rate of fall, while at the periphery F rises

¹ We are indebted to Dr. George Wald for the opportunity to consider his results in advance of their publication.

during dark adaptation; at intermediate locations it goes through a minimum, an effect not detected in a night-blind subject.

The conception here arrived at, of the essential proportionality of critical F to magnitude of sensory effect, has certain important consequences when combined with the intensity discrimination theory of response to flicker. These we shall attempt to utilize in a later paper.

If this mode of dissection of the rod and cone contributions to the flicker response curve is correct in principle, then it will be observed that there is provided a case in which it might be argued by some that the coming into action of retinal cones progressively "inhibits" the effective performance of rods—one type of "retinal interaction." But the argument would be pointless because (1) the cone curve, at sufficiently high intensities, also bends over; and (2) the effect is less obscurely accounted for by consideration of the basis of recognition of flicker.

There are two rather different tests which can be made of predictions called for by our view. (1) The flicker curve for a cone-free human subject should be found to rise to a low maximum and then to decline. (2) It should be possible to find fishes in which the separation of the rod and cone curves is in one way or another more pronounced, so that several forms of transition to the cone section of the flicker curve could be measured. The data on several human subjects (Hecht and Verrijp (1932-33 *a*)) suggest individual differences. Fresh water teleosts have the advantage for this purpose that hybridization is possible with suitably selected material. A genetic test of the basis for differences in flicker curves could then be made in the manner employed for the analysis of geotropic response (Crozier and Pincus (1931-32 *b*)). It is to be expected also that by this procedure the capacity to exhibit variation in critical intensity for response to flicker may be submitted to functional dissection, since this must be in part determined, as to absolute amount, by properties of the reaction system of the species and in further part by the active mechanism of discrimination of flicker.

VI

SUMMARY

The curve connecting mean critical illumination (I_m) and flicker frequency (F) for response of the sunfish *Lepomis* (*Enneacanthus*

gloriosus) to flicker is systematically displaced toward lower intensities by raising the temperature. The rod and cone portions of the curve are affected in a similar way, so that (until maximum F is approached) the shift is a nearly constant fraction of I_m for a given change of temperature. These relationships are precisely similar to those found in the larvae of the dragonfly *Anax*. The modifications of the variability functions are also completely analogous. The effects found are consistent with the view that response to flicker is basically a matter of discrimination between effect of flashes of light and their after effects,—a form of intensity discrimination. They are not consistent with the stationary state formulation of the shape of the flicker curve.

An examination of the relationships between the cone portion and the rod portion of the curves for the sunfish suggests a basis for their separation, and provides an explanation for certain "anomalous" features of human flicker curves. It is pointed out how tests of this matter will be made.

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